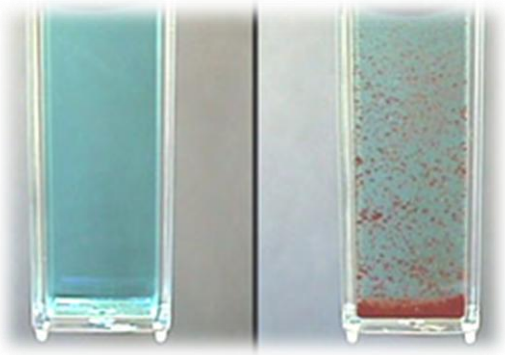
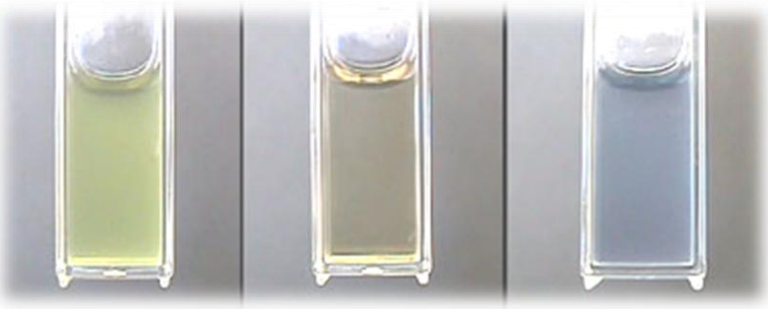


Qualitative analysis of Carbohydrates I

By : Amal Alamri



carbohydrates

The term carbohydrates are generally applied to the group of ***polyhydroxy aldehydes*** or ***ketones*** commonly known as sugars.

Carbohydrates are produced from CO2 and H2O by plants through the process of **photosynthesis**.

Carbohydrates are the major food supply and energy source for the people of the world.

Despite the major utilization of carbohydrates for **energy** ,only a small amount is stored in the body. The average adult reserve is about 370 g **stored** mainly as **liver and muscle glycogen**.

Classification of Carbohydrates

I-According to chemical composition:

Simple and complex

II-According to number of units:

Mono- Di- Oligo- Poly- saccharide

III- According to position of carbonyl group in mono saccharid:

Aldoses and Ketoses

IV- According to the number of carbon atoms in mono saccharid:

triose(3),tetrose(4), pentose(5),hexose(6),heptose(7)

I-According to chemical composition:

1) **Simple** carbohydrates, often called monosaccharides or simple sugars, contain **one** saccharide unit.

2) **Complex** carbohydrates are those containing **more than one** saccharide group.

II-According to number of units:

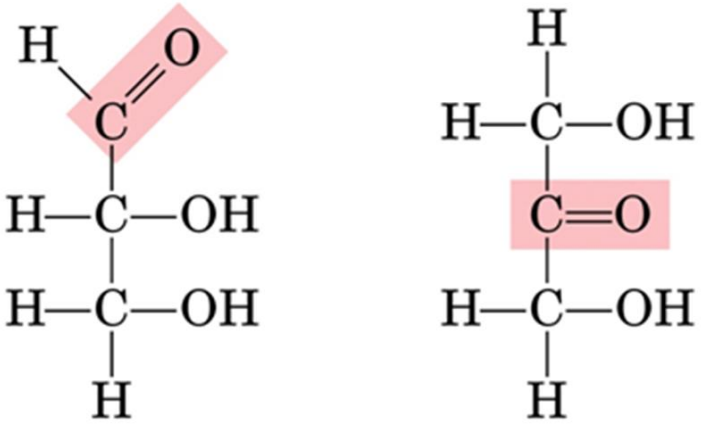
- **Monosaccharides** contain **one** monosaccharide unit.
- **Disaccharides** contain **two** monosaccharide units.
- **Oligosaccharides** contain **3-6** monosaccharide units.
- **Polysaccharides** can contain over **7 or more** monosaccharide units.

- Complex carbohydrates can be broken down into smaller sugar units through a process known as **hydrolysis**.

III- According to position of carbonyl group in mono saccharid:

- **An aldose** contains **terminal aldehyde** group in addition to R group containing -OH.

- **A ketose :**contains a carbonyl group **attached to two R** groups having one or more hydroxyl groups.



Aldose Ketose

IV- According to the number of carbon atoms in mono saccharid:

They can be classified by the number of carbon atoms they contain; pentoses (5 carbons) and hexoses (6 carbons) are the most common.

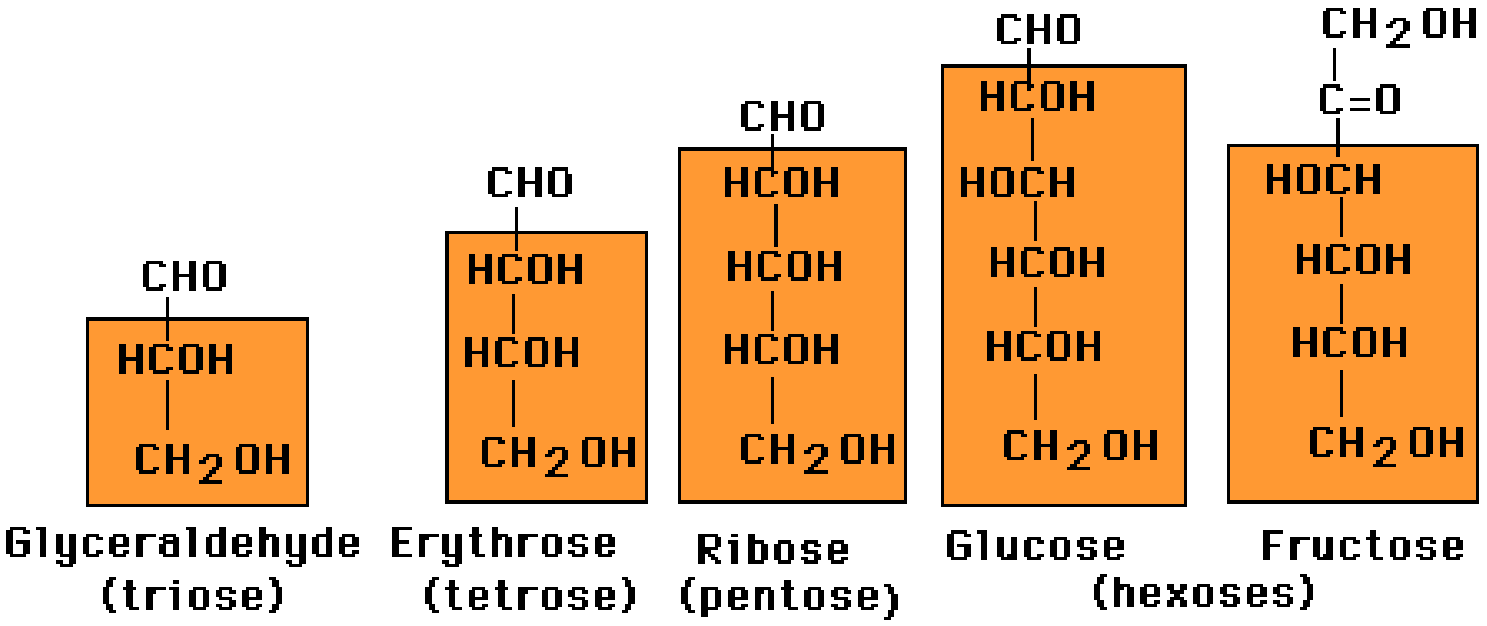
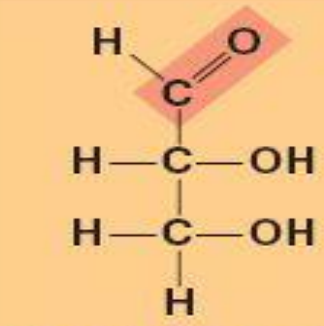
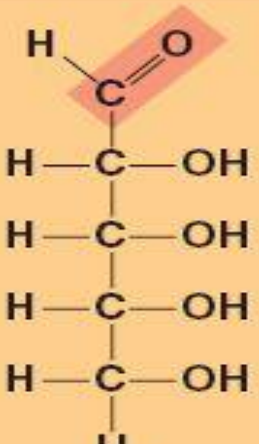
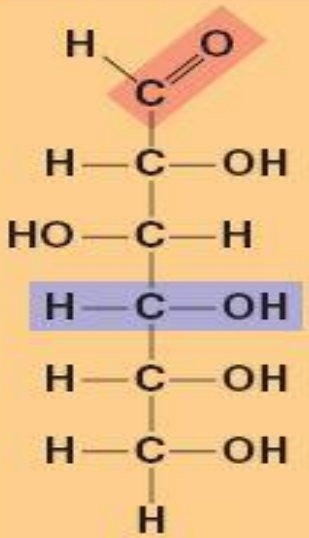
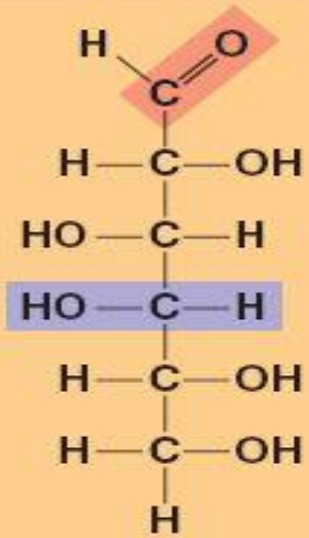
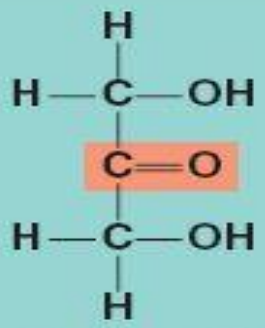
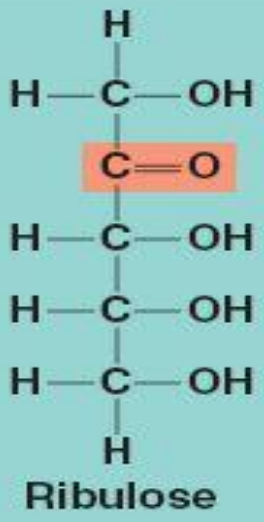
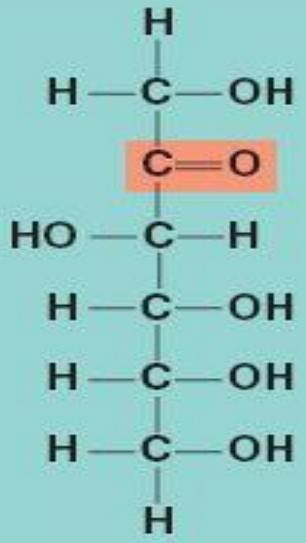


Fig. Monosaccharides with various numbers of carbon atoms.

	Triose sugars ($C_3H_6O_3$)	Pentose sugars ($C_5H_{10}O_5$)	Hexose sugars ($C_6H_{12}O_6$)	
Aldoses	 Glyceraldehyde	 Ribose	 Glucose	 Galactose
Ketoses	 Dihydroxyacetone	 Ribulose	 Fructose	

Physical Properties of Carbohydrates:

Solubility:

Monosaccharide and disaccharide can be dissolved freely in water because water is a **polar substance**, while **polysaccharide cannot** be dissolved easily in water, because, it has **high molecular weight** , which give **colloidal** solutions in water soluble.

Monosaccharide and disaccharide dissolved in water

Starch do not dissolved easily in water

Chemical Properties of Carbohydrates:

1- Molisch Test: specific for carbohydrates.

2- Benedict's Test: presence of reducing sugars

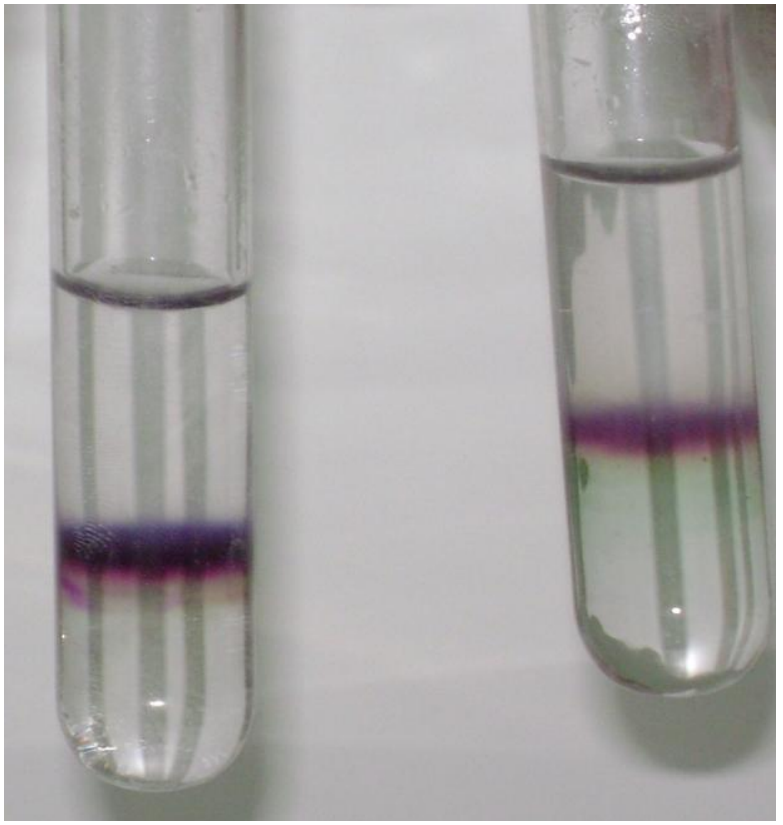
3- Barfoed's Test: distinguish between reducing monosaccharides, reducing disaccharides and non reducing disaccharides.

4- Bial's Test: distinguish between pentose and hexose monosacharides

5-Seliwanoff's Test: distinguish between aldoses and ketoses

Molisch Test:

Specific for all carbohydrates, Monosaccharide gives a rapid positive test, Disaccharides and polysaccharides react slower.



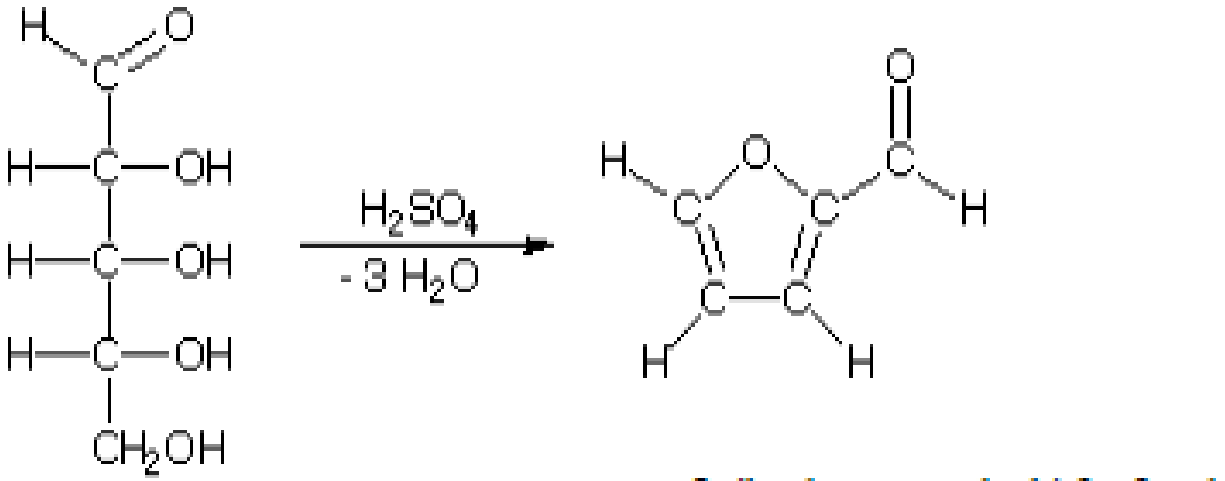
Objective:

To identify the carbohydrate from other macromolecules lipids and proteins.

Principle:

The test reagent **dehydrates pentoses** to form furfural and **dehydrates hexoses** to form 5-hydroxymethyl furfural.

The furfurals further react with α-naphthol present in the test reagent to produce a purple product.



Glucose

5-(hydroxymethyl)furfural

Method:

- 2ml of a sample solution in a test tube.
- 4 drops of the **Molisch reagent (a solution of α-napthol in 95% ethanol) is added**.
-The solution is then poured slowly into a tube containing 2ml of concentrated sulfuric acid so that two layers form, producing **violet ring appear** as liaison between the surface separations.

Tube	Observation
Glucose	
Lactose	
Starch	

Benedict's Test:

Objective:

To distinguish between reducing and non-reducing sugars

Benedict's reagent is used as a test for the presence of reducing sugars.

All monosaccharides are reducing sugars; they all have a free reactive carbonyl group.

Some disaccharides have exposed carbonyl groups and are also reducing sugars.

Other disaccharides such as sucrose are non-reducing sugars and will not react with Benedict's solution.
Starch are also non-reducing sugars

Principle:

The **copper sulfate (CuSO4)** present in Benedict's solution reacts with **electrons** from the **aldehyde or ketone group** of the reducing sugar. Reducing sugars are oxidized by the copper ion in solution to form a carboxylic acid and a reddish precipitate of copper

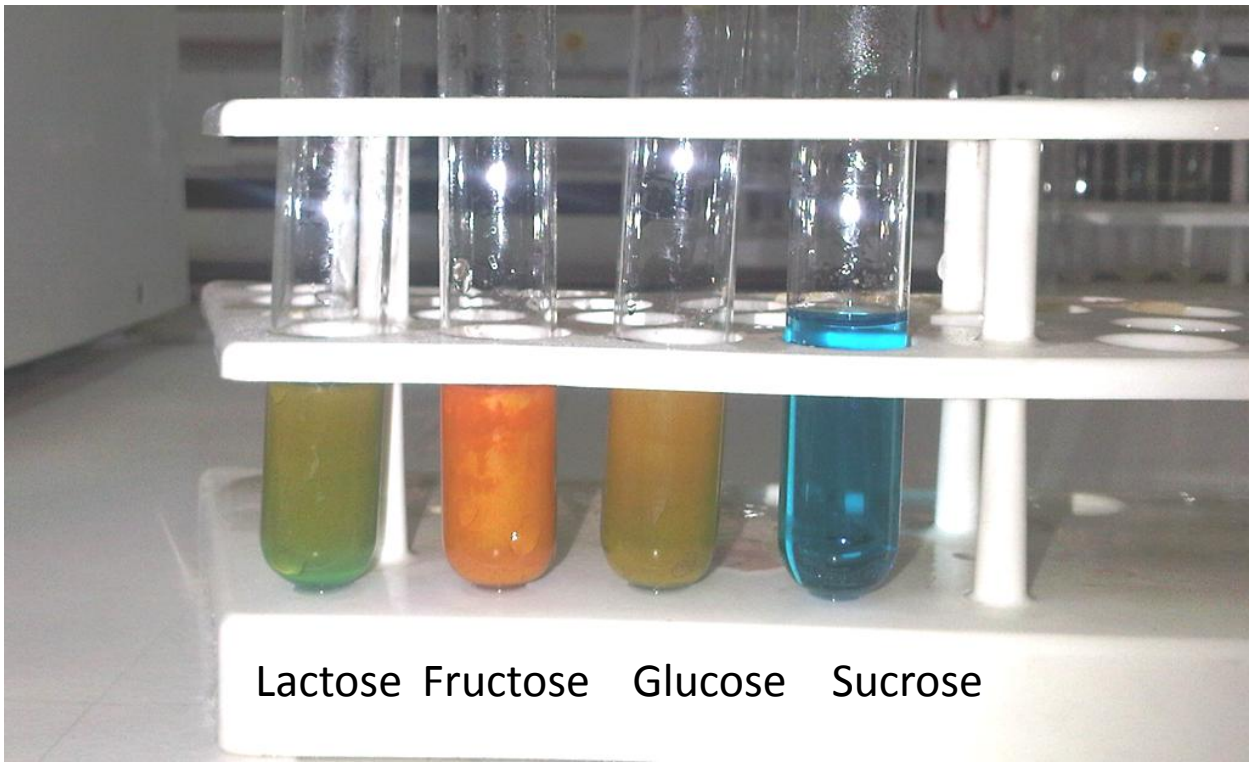
(I) oxide.

$$CuSO_4 \longrightarrow Cu^{++} + SO_4^{--}$$

$$2\,Cu^{++} + \text{Reducing Sugar} \longrightarrow Cu+$$

(electron donor)

$$Cu^{+} \longrightarrow Cu_2O \text{ (precipitate)}$$



Method:

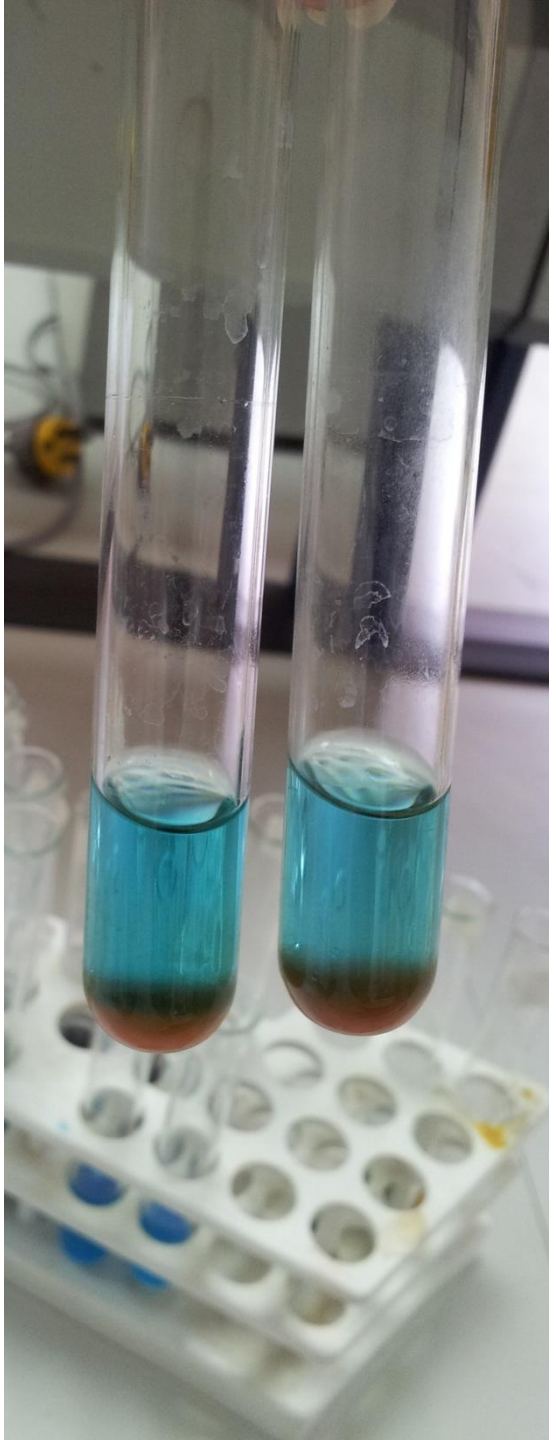
- 1 ml of a sample solution is placed in a test tube.
- 2 ml of Benedict's reagent is added.
- The solution is then **heated in a boiling** water bath for **three minutes**.

A positive test is indicated by: The formation of a reddish precipitate within 3 minutes

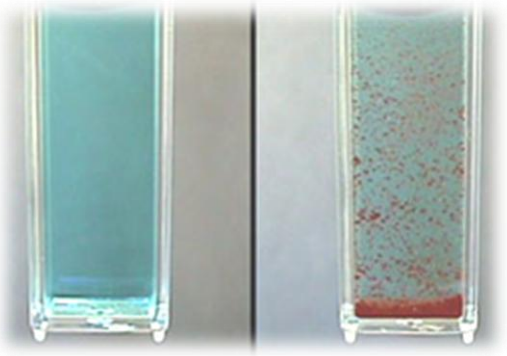
Tube	Observation
Glucose	
Fructose	
Sucrose	
Lactose	

Barfoed's Test:

This test is performed to distinguish between reducing monosaccharides, reducing disaccharides .

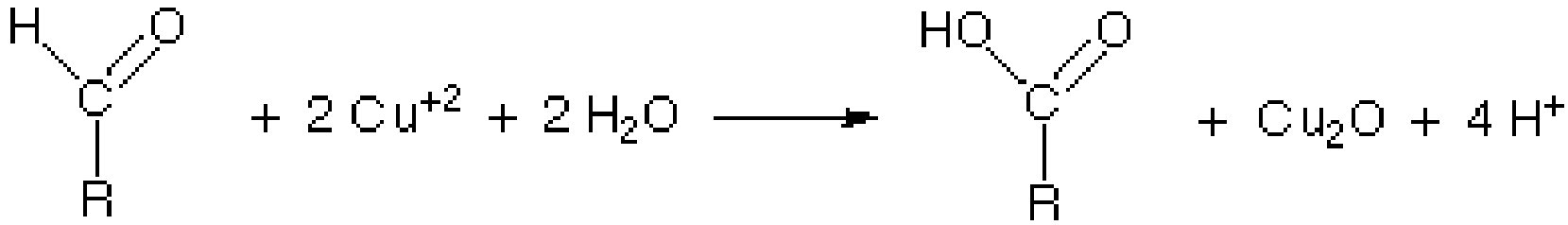


Principle:



Barfoed's test used copper (II) ions in a slightly acidic medium , reducing monosaccharides are oxidized by the copper ion in solution to form a **carboxylic acid and a reddish precipitate of copper (I) oxide** within three minutes**.**

Reducing disaccharides undergo the same reaction, but do so **at a slower rate.** The nonreducing sugars give negative result.



Method:

- 1 ml of a sample solution in a test tube.
- 3 ml of **Barfoed's reagent (a solution of cupric acetate and acetic acid.**
- Heat the solution in a boiling water bath for three minutes.

Tube	Observation
Glucose	
Fructose	
Sucrose	

Bial's Test:

This test is used to distinguish between **pentose** and hexose monosacharides.

Principle:

Bial’s test uses conc. HCl as a *dehydrating* acid and orcinol + traces of ferric chloride as condensation reagent.

The test reagent dehydrates pentoses to form furfural.

Furfural further reacts with orcinol and the iron ion present in the test reagent to produce a bluish or green product, while hexoses yield muddy-brown to grey condensation product.

Method:

1- Put 2 ml of a sample solution in a test tube.

2- Add 2 ml of Bial's reagent (a solution of orcinol, HCl and ferric chloride) to each tube.

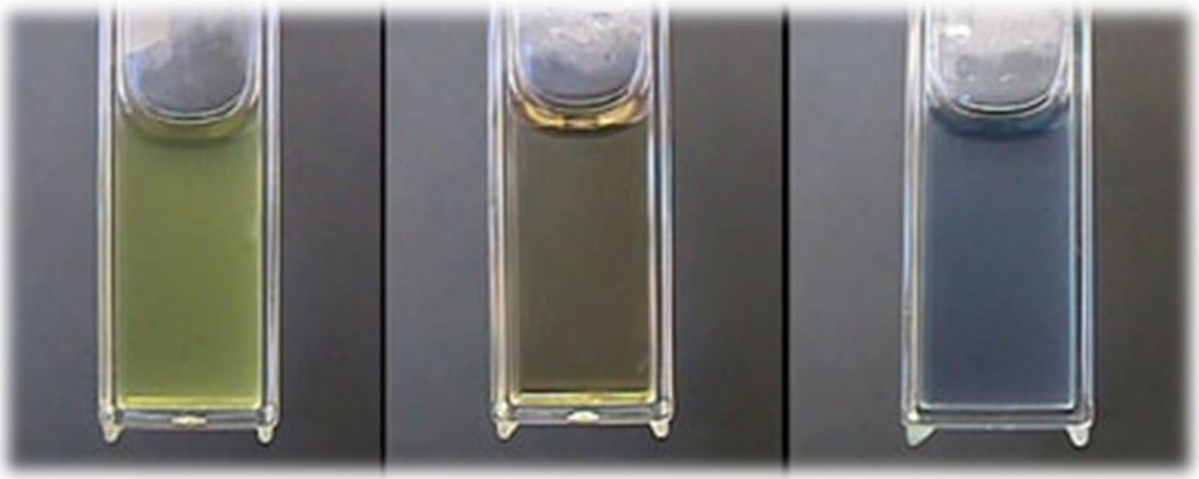
3-Heat the tubes gently in hot water bath.

- If the color is not obvious, more water can be added to the tube.

Tube	Observation
Glucose	
Fructose	
Ribose	

A positive test is indicated by the formation of a bluish product.

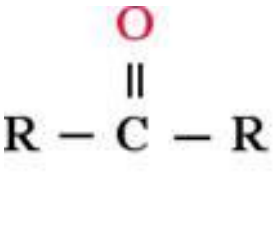
All other colors indicate a negative result for pentoses. Note that hexoses generally react to form green, red, or brown products.



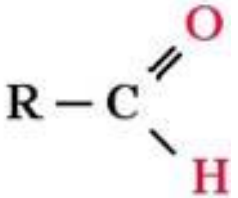
Negative Negative positive

Seliwanoff's Test:

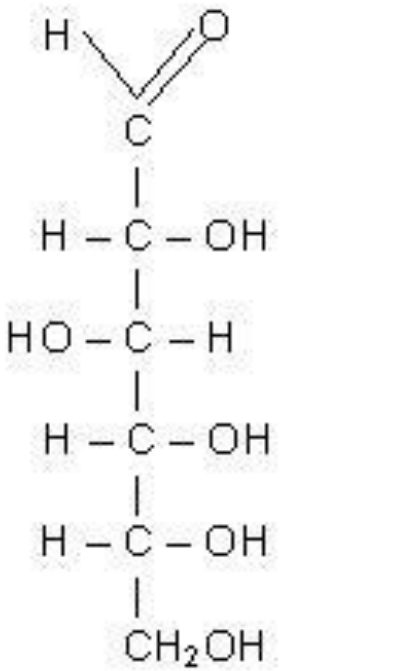
This test is used to distinguish between **aldoses** (like glucose) and **ketoses** (like fructose).



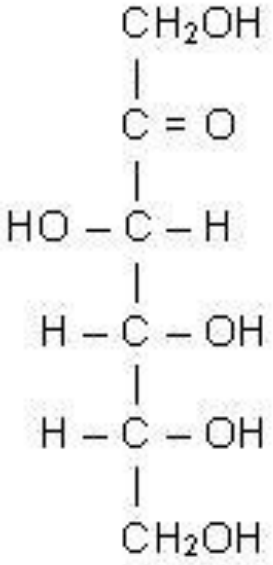
Ketone Group



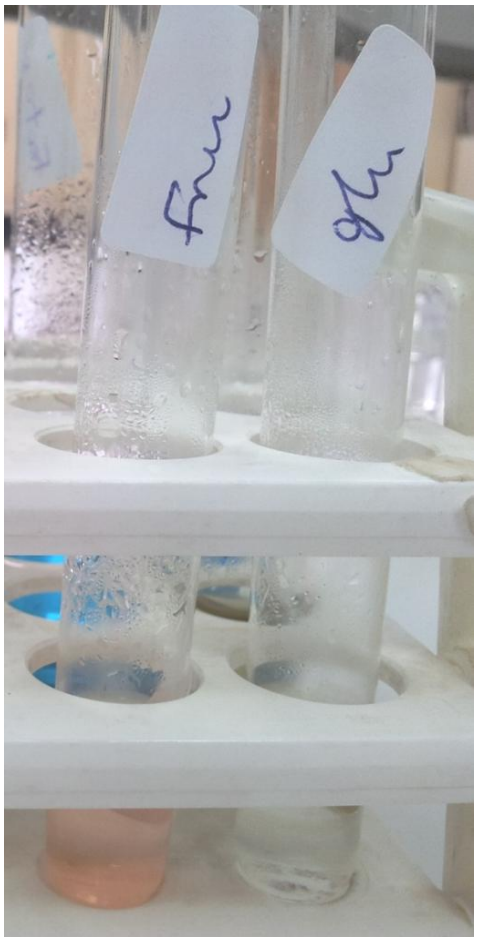
Aldehyde Group



Glucose (an aldohexose)



fructose
(a ketohexose)

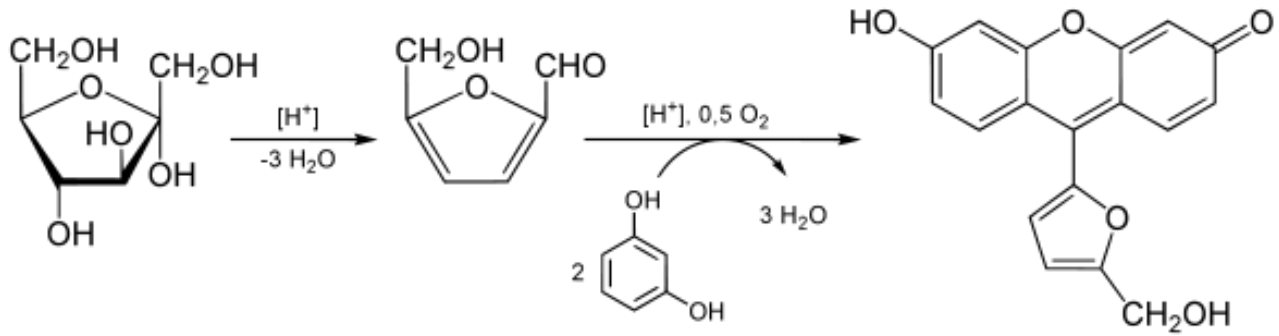


Principle:

Seliwanoff's Test uses 6M HCl as dehydrating agent and resoncinol as condensation reagent.

The test reagent dehydrates ketohexoses to form 5-hydroxymethylfurfural. 5-hydroxymethylfurfural further condenses with resorcinol present in the test reagent to produce a cherry red product .

Aldohexoses react to form the same product, but do **so more slowly giving yellow to faint pink** color.



Method:

- 1 ml of a sample solution is placed in a test tube.
- 2.5ml of Seliwanoff's reagent
- The solution is then heated in a boiling water bath for 5 minutes.

Result:

Tube	Observation
Glucose	
Fructose	

THANK YOU